Int J Clin Exp Med 2015;8(6):8854-8859
www.ijcem.com /ISSN:1940-5901/IJCEM0007121

Original Article

Apigenin attenuates acute myocardial infarction of rats via the inhibitions of matrix metalloprotease-9 and inflammatory reactions

Hong Du, Jie Hao, Fan Liu, Jingchao Lu, Xiuchun Yang

Department of Cardiology, Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China

Received February 15, 2015; Accepted April 23, 2015; Epub June 15, 2015; Published June 30, 2015

Abstract: Acute myocardial infarction (AMI) is the myocardial necrosis caused by coronary artery acute and persistent ischemia and hypoxia. Matrix metalloprotease-9 (MMP-9) plays an important role in a series of process of occurrence and development of AMI. Inflammatory reaction plays the key role in all kinds of damage factors in AMI. Apigenin (API) has effectively restrained the activity of MMP-9, anti-inflammatory and hepatic fat oxidizing properties. API significantly improved AMI of rats through inhibiting MMP-9 and inflammatory reactions in a few recent studies. Our investigation detected the infarct size of AMI rats, casein kinase (CK), the MB isoenzyme of creatine kinase (CK-MB) and lactate dehydrogenase (LDH) and cardiac troponin T (cTnT) activities in AMI rats were also analyzed with commercial kits. Additionally, Nuclear factor kappa B (NF-κB), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) levels of whole bloods of AMI rats were also detected using commercial kits. Next, MMP-9 protein of cardiac in AMI rats was measured with gelatin zymography assays. Finally, caspase-3 and caspase-9 activities in AMI rats were analyzed with commercial kits. In the present study, our work indicated API might significantly reduce the infarction size of AMI rat. It was shown that the treatment of API could decrease the expression of MMP-9 level and reduce the activities of NF-κB, TNF-α, IL-1β and IL-6 in AMI rats. Next, API treatment could reduce caspase-3 and caspase-9 activities and decrease cellular apoptosis of AMI rats. Our findings concluded that API ameliorates acute myocardial infarction of rats via inhibiting MMP-9 and inflammatory reactions.

Keywords: Apigenin, acute myocardial infarction, MMP-9, inflammatory, apoptosis

Introduction

It was well established that acute myocardial infarction (AMI) is caused by persistent ischemia and hypoxia, finally leading to many clinical cases of severe and persistent pain behind the sternum [1]. It is a very common disorder in Western countries [2]. In recent years, there is a rising trend in China. It was estimated that the new morbidity quantity in each year was increased to at least 500,000 [3].

It is well known that matrix Metalloproteases (MMPs) contain at least 26 members and can adjust and control the synthesis and degradation of extracellular matrix. Prior work illustrated that it was involved in the reconstruction of ventricular [4]. MMP-9 is the important members of matrix metalloproteinases, which is secreted by vascular smooth muscle cells and the expression of them is significantly increased in atherosclerotic plaque, contributing to the pathogenesis of AMI [5].

Inflammatory reaction is closely involved in the generation of AMI. Under normal conditions, inflammatory cells and vascular endothelial cells do not attach or hardly attach. But after stimulation by inflammatory factor, adhesion molecule can be quickly activated and help the inflammatory cell to be attached and accumulated in the endothelial cells, subsequently releasing the inflammatory mediators to damage the endothelial cells [6]. Three kinds of selectins of adhesion molecules were found to be related to the adhesion and rolling of inflammatory cells in vascular endothelial and the accumulation of damaged region [7].

It was previously reported that API possessed diverse pharmacological actions including antitumor, anti-oxidative stress, anti-DNA damage

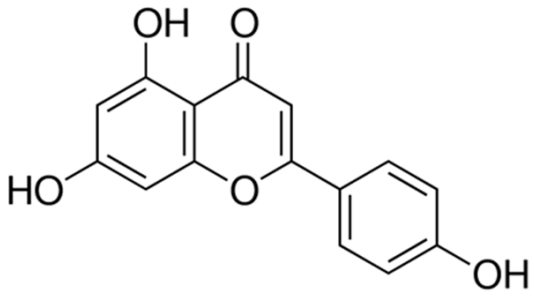

Figure 1. The chemical structure of API.

and so on [8]. Furthermore, API was found to remarkably suppress metastasis via MMP-9 pathway in ovarian tumor model [9]. However, up to date, there is no relevant publication on the effect of API against AMI. Our present work aimed to evaluate the protective potential of API against AMI and further figure out its potential molecular mechanism.

Materials and methods

Experimental animals

Sixty male Wistar rats, weighing 220-250 g, were supplied from the Experimental Animal Center of Second Hospital of Hebei Medical University. All surgical procedures have been approved by Second Hospital of Hebei Medical University and performed in strict accordance with the related ethical standards.

Drug administration

API (Sigma, with a purity of 95%) was dissolved in physiological saline. Casein kinase (CK), the MB isoenzyme of creatine kinase (CK-MB), lactate dehydrogenase (LDH) and cardiac troponin T (cTnT) commercial kits were from Sangong Biotech (Shanghai, China). Nuclear factor kappa B (NF-κB), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) commercial kits were purchased from JianCheng Bioengineering (Nanjing, China). BCA protein assay kit was supplied from Tiangen (Beijing, China). Caspase-3 and Caspase-9 Activities Assay Kit were bought from Sangong Biotech (Shanghai, China).

Preparation of acute myocardial infarction and group design

AMI model was prepared as previously described [10]. Successful establishment of all rats AMI models was verified by regional cyanosis of myocardial surface and represented by ST-segment elevation. All experimental rats were randomly divided into sham group (n = 10), Vehicle group (n = 10) and API treatment group (n = 10) (various does of API: 10 mg/kg, 20 mg/kg and 40 mg/kg, respectively). Rats of sham group were treated identically except for no ligation. Rats of vehicle group were administrated with the equal volume of physiological saline. Physiological saline and API were injected once a day. Rats were operated on by occlusion of coronary artery after the last administration at 30 minutes.

Infarct size measurement

After ligation for 6 h, the hearts from different groups were carefully dissected and 2 mm thick sections was sliced for infarct size measurement. Infarct size of rats was determined with 1% 2, 3, 5-triphenyltetrazolium chloride (1.5%; Sigma-Aldrich Co.) at 37°C for 30 min in the dark [11, 12]. The area of heart without color was deemed as the ischemic heart muscles, but the area with brick red was considered as normal myocardium. The infarct size area was calculated by the volume and weight as a percentage of the left ventricle.

Determinations of CK, CK-MB, LDH and cTnT

Serum samples of rats were obtained from vena cava after ligation for 6 h. The CK, CK-MB, LDH and cTnT activities in rats were detected by respective commercial kits (Sangong Biotech, Shanghai, China).

Measurement of NF-κB, TNF-α, IL-1β and IL-6 levels

After 6 h ligation, whole bloods of rats from different groups were collected and a commercial ELISA kit was employed to measure the serum levels of NF-κB, TNF-α, IL-1β and IL-6 in rats following the manufacturer's instruction.

Measurement of MMP-9

After API treatment, MMP-9 activities were detected by gelatin zymography assays. For short, 50 μg samples were absorbed into the new centrifuge tube and then an equal volume of sodium dodecyl sulfate (SDS) sample buffer were added into the centrifuge tube. The miscible liquids were subjected to 10% SDS-PAGE

Table 1. The activities of CK, CK-MB, and LDH, the level of cTnT in a rat model of AMI (mean ± S.D., n = 10)

Groups	CK (U/mL)	CK-MB (IU/L)	LDH (U/L)	cTnT (U/mL)
Sham	0.21 ± 0.02	83.11 ± 7.11	1713.11 ± 311.27	0.06 ± 0.02
Vehicle	0.78 ± 0.05**	212.35 ± 7.19**	5747.39 ± 437.62**	0.67 ± 0.04**
API (10 mg/kg)	0.38 ± 0.02##	113.15 ± 7.48##	3135.26 ± 335.62#	0.23 ± 0.05##
API (20 mg/kg)	0.31 ± 0.04##	95.03 ± 7.29##	2678.84 ± 316.26##	0.19 ± 0.04##
API (40 mg/kg)	0.29 ± 0.03##	85.66 ± 8.23##	2367.89 ± 324.21##	0.16 ± 0.02##

**P<0.01 vs. sham group, #P<0.05, ##P<0.01 vs. vehicle group. Sham, sham-operated; Vehicle, vehicle-treated; API (10 mg/kg)-treated; API, (20 mg/kg)-treated; API, (40 mg/kg)-treated groups.

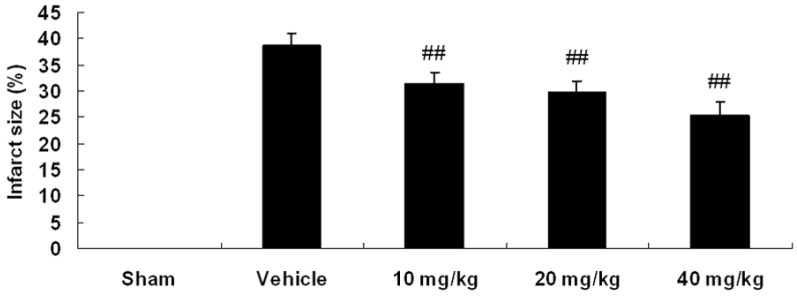

Figure 2. API decreases infarct size in a rat model of AMI (n = 10, mean ± S.D.). ##P<0.01 versus vehicle group.

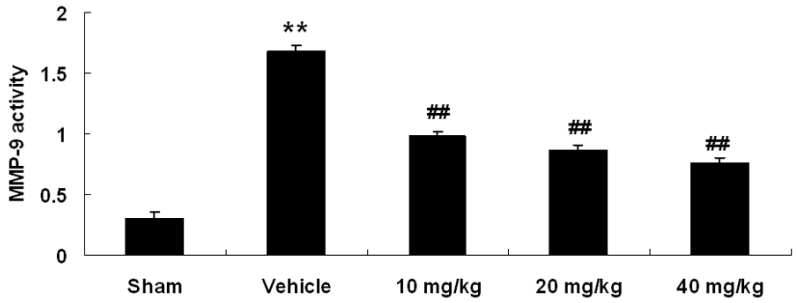

Figure 3. API inhibits MMP-9 in a rat model of AMI (n = 10, mean ± S.D.). MMP-9 activity was dose-dependently reduced after exposure to API for 24 h by Gelatin zymography assays. **P<0.01 versus sham group; ##P<0.01 versus vehicle group.

electrophoresis gel impregnated with 0.1% gelatin. The gel was washed three times for 20 minutes in 2.5% Triton X-100 to remove SDS after electrophoresis. The gel was incubated in a reaction buffer at 37°C for 12 h. Then, the gel was stained with 0.05% and finally Coomassie brilliant blue R-250 was employed to stain the gels.

Caspase-3 and caspase-9 activities assay

In order to detect the apoptotic state during AMI, caspase-3 and caspase-9 activities were measured by corresponding Assay Kits (Sangong Biotech, Shanghai, China). The data was expressed as the percentage of the control group.

Statistics

Data were expressed as means ± S.D. and analyzed by SPSS 17.0 software. Differences between experimental groups were analyzed using ANOVA analysis. A p value of less than 0.05 were deemed statistically significant.

Results

API suppresses the activities of CK, CK-MB, and LDH, the level of cTnT in a rat model of AMI

The chemical structure of API was displayed in **Figure 1**. It was obviously found that the activities of CK, CK-MB, LDH and cTnT were significant increased in the serum, compared with the sham controls (**Table 1**). When treatment with API (10, 20 and 40 mg/kg) for 24 h, the remarkably reduced levels of CK, CK-MB, LDH and cTnT were observed in serum of AMI rat model, compared to that in the vehicle group (**Table 1**).

API decreases the Infarct size in AMI rat model

We then investigated whether the effect of API on the infarction size of AMI rat. After treatment with API (10, 20 and 40 mg/kg) for 24 h, the

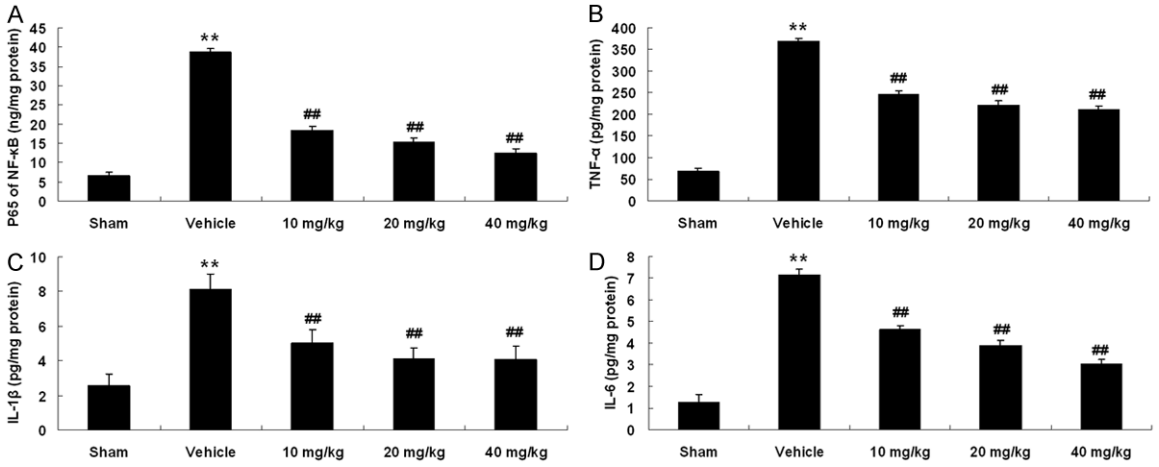

Figure 4. API reduces the inflammatory responses in a rat model of AMI (n = 10, mean ± S.D.). (A-D) indicated the activities of NF-κB, TNF-α, IL-1β and IL-6, respectively, in different groups. **P<0.01 versus sham group; ##P<0.01 versus vehicle group. Sham, sham-operated; Vehicle, vehicle-treated; 10 mg/kg, API (10 mg/kg)-treated, 20 mg/kg, API (20 mg/kg)-treated, 40 mg/kg, API (40 mg/kg)-treated.

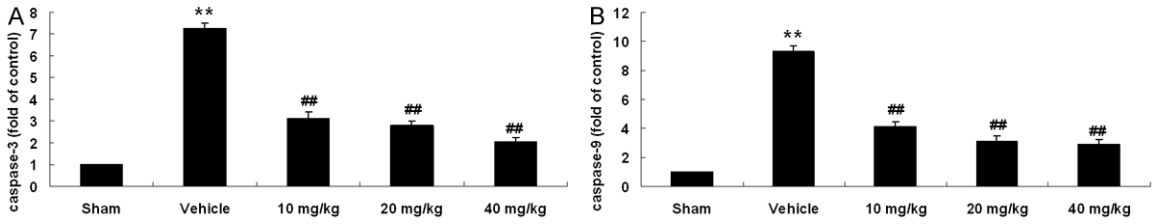

Figure 5. API inhibits the cellular apoptosis in a rat model of AMI (n = 10, mean ± S.D.). (A, B) Displayed the activities of caspase-3 and caspase-9 respectively, in different groups. **P<0.01 versus sham group; ##P<0.01 versus vehicle group. Sham, sham-operated; Vehicle, vehicle-treated; 10 mg/kg, API (10 mg/kg)-treated, 20 mg/kg, API (20 mg/kg)-treated, 40 mg/kg, API (40 mg/kg)-treated.

infarction size were significantly reduced, compared to the vehicle group (**Figure 2**).

API inhibits MMP-9 in a rat model of AMI

Next we aimed to analyze whether API might affect MMP-2 production in AMI rat model. We found that the activity of MMP-9 in vehicle group was markedly increased, compared to the sham group (**Figure 3**). However, API treatment dose-dependently reversed this phenomenon in rats with AMI, compared to the vehicle group.

API suppresses the activities of TNF-α, IL-1β, IL-6 and NF-κB in AMI rat model

It was remarkably found that inflammatory cytokines including NF-κB, TNF-α, IL-1β and IL-6 were evidently activated during AMI, as illustrated in **Figure 4A-D**. Treatment with API (10, 20 and 40 mg/kg) for 24 h could obviously reduce the inflammatory mediators (NF-κB, TNF-α, IL-1β and IL-6), compared to the vehicle group.

API inhibits the activities of caspase-3 and caspase-9 in a rat model of AMI

To confirm the protection of API against AMI, caspase-3 and caspase-9 activities were analyzed in our present study. We found that caspase-3 and caspase-9 activities in vehicle group were evidently increased, compared to the sham group (**Figure 5A**, **5B**). It was noted that caspase-3 and caspase-9 activities were remarkably suppressed in API-treated AMI group.

Discussion

The major findings of our present work depicted that API, the well-known medicinal herb, has effective role against AMI in rats and further

illustrated its protection might be associated with inhibiting MMP-9 activity and inflammatory reactions.

MMPs is normally secreted by myocardial cells, vascular endothelial cells, smooth muscle cells, foam cells and other cells, including the protease family that depends on Ca^{2+} and Zn^{2+}. Current work shows that MMPs is the protease to adjust and control extracellular matrix, which can promote the degradation of ECM, accelerate the progression of atheromatous plaque and lead to the formation of unstable plaque [13, 14]. MMP-9 can reflect the status of atherosclerotic plaque and plays an important role in a series of process of generation of AMI [15]. Our study disclosed that API treatment significantly suppressed MMP-9 activity in rats subjected to AMI. In fact, prior work illustrated that API could inhibit metastasis of orthotopic ovarian tumor and down-regulate MMP-9, which was in agreement with our findings [9].

After AMI, the inflammatory factors such as TNF-α, IL-1β and IL-6 were reported to be elevated and their rising degree was closely related to the disease severity and prognosis of the patients [16]. Meanwhile, in atherosclerosis, NF-κB could activate TNF, IL-1, IL-8 and other inflammatory factors [17]. Therefore, activation of NF-κB is also likely to be participated in the pathogenesis of AMI. Our study revealed that API could obviously reduce the activities of NF-κB, TNF-α, IL-1β and IL-6 in AMI rats. It was previously found that API evidently alleviated cutaneous inflammation in murine models of acute dermatitis [18]. Besides, API was also found to weaken inflammatory reaction following UVB-induced skin inflammation [19]. In the meantime, our present work also showed that API treatment could reduce caspase-3 and caspase-9 activities in AMI rat models. Consistently, API was also shown to have protection against liver ischemia-reperfusion injury in rats [20].

In conclusion, it was for the first time to delineate that API could alleviate AMI injury and decrease the infarction size in AMI rats. Meanwhile, further investigation disclosed that the protection of API against AMI rat models was linked with inhibiting MMP-9 activity and inflammatory reactions.

Disclosure of conflict of interest

None.

Address correspondence to: Fan Liu, Department of Cardiology, Second Hospital of Hebei Medical University, Shijiazhuang 050000, China; Fax: +86-311-66003972; Tel: +86-311-66003972; E-mail: fanliudr2015@163.com

References

[1] Bradshaw PJ, Trafalski S, Hung J, Briffa TG and Einarsdottir K. Outcomes after first percutaneous coronary intervention for acute myocardial infarction according to patient funding source. BMC Health Serv Res 2014; 14: 405.

[2] Pazoki R, de Jong JS, Marsman RF, Bruinsma N, Dekker LR, Wilde AA, Bezzina CR and Tanck MW. SNPs identified as modulators of ECG traits in the general population do not markedly affect ECG traits during acute myocardial infarction nor ventricular fibrillation risk in this condition. PLoS One 2013; 8: e57216.

[3] Wang DW, Zhang Y, Yao JM and Xiao ZB. Surgical treatment of a ventricular aneurysm in a patient with essential thrombocythemia complicated by acute myocardial infarction: A case report. Exp Ther Med 2014; 7: 267-269.

[4] Konnecke H and Bechmann I. The role of microglia and matrix metalloproteinases involvement in neuroinflammation and gliomas. Clin Dev Immunol 2013; 2013: 914104.

[5] Squire IB, Evans J, Ng LL, Loftus IM and Thompson MM. Plasma MMP-9 and MMP-2 following acute myocardial infarction in man: correlation with echocardiographic and neurohumoral parameters of left ventricular dysfunction. J Card Fail 2004; 10: 328-333.

[6] Wollenweber T, Roentgen P, Schafer A, Schatka I, Zwadlo C, Brunkhorst T, Berding G, Bauersachs J and Bengel FM. Characterizing the inflammatory tissue response to acute myocardial infarction by clinical multimodality noninvasive imaging. Circ Cardiovasc Imaging 2014; 7: 811-818.

[7] Halladin NL, Ekelof S, Jensen SE, Aaroe J, Kjaergaard B, Heegaard PM, Lykkesfeldt J, Rosenberg J and Gogenur I. Melatonin does not affect oxidative/inflammatory biomarkers in a closed-chest porcine model of acute myocardial infarction. In Vivo 2014; 28: 483-488.

[8] Wang QQ, Cheng N, Yi WB, Peng SM and Zou XQ. Synthesis, nitric oxide release, and alpha-glucosidase inhibition of nitric oxide donating apigenin and chrysin derivatives. Bioorg Med Chem 2014; 22: 1515-1521.

[9] He J, Xu Q, Wang M, Li C, Qian X, Shi Z, Liu LZ and Jiang BH. Oral administration of apigenin inhibits metastasis through AKT/P70S6K1/MMP-9 pathway in orthotopic ovarian tumor model. Int J Mol Sci 2012; 13: 7271-7282.

[10] Yu HP, Liu FC, Tsai YF and Hwang TL. Osthole attenuates hepatic injury in a rodent model of

trauma-hemorrhage. PLoS One 2013; 8: e65916.
[11] Hoda MN, Li W, Ahmad A, Ogbi S, Zemskova MA, Johnson MH, Ergul A, Hill WD, Hess DC and Sazonova IY. Sex-independent neuroprotection with minocycline after experimental thromboembolic stroke. Exp Transl Stroke Med 2011; 3: 16.
[12] Hoda MN, Siddiqui S, Herberg S, Periyasamy-Thandavan S, Bhatia K, Hafez SS, Johnson MH, Hill WD, Ergul A, Fagan SC and Hess DC. Remote ischemic perconditioning is effective alone and in combination with intravenous tissue-type plasminogen activator in murine model of embolic stroke. Stroke 2012; 43: 2794-2799.
[13] Wang KF, Huang PH, Chiang CH, Hsu CY, Leu HB, Chen JW and Lin SJ. Usefulness of plasma matrix metalloproteinase-9 level in predicting future coronary revascularization in patients after acute myocardial infarction. Coron Artery Dis 2013; 24: 23-28.
[14] Periasamy S, Mo FE, Chen SY, Chang CC and Liu MY. Sesamol attenuates isoproterenol-induced acute myocardial infarction via inhibition of matrix metalloproteinase-2 and -9 expression in rats. Cell Physiol Biochem 2011; 27: 273-280.
[15] Dai F, Xiao GL and Pan J. [Correlation between serum MMP-2 and MMP-9 in patients with acute myocardial infarction before and after PCI]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2009; 25: 716-718.
[16] Stein A, Mohr F, Laux M, Thieme S, Lorenz B, Cetindis M, Hackl J, Groha P, Demetz G, Schulz S, Mehilli J, Schomig A, Kastrati A and Ott I. Erythropoietin-induced progenitor cell mobilisation in patients with acute ST-segment-elevation myocardial infarction and restenosis. Thromb Haemost 2012; 107: 769-774.
[17] Turner NA, Das A, Warburton P, O'Regan DJ, Ball SG and Porter KE. Interleukin-1alpha stimulates proinflammatory cytokine expression in human cardiac myofibroblasts. Am J Physiol Heart Circ Physiol 2009; 297: H1117-1127.
[18] Man MQ, Hupe M, Sun R, Man G, Mauro TM and Elias PM. Topical apigenin alleviates cutaneous inflammation in murine models. Evid Based Complement Alternat Med 2012; 2012: 912028.
[19] Byun S, Park J, Lee E, Lim S, Yu JG, Lee SJ, Chen H, Dong Z, Lee KW and Lee HJ. Src kinase is a direct target of apigenin against UVB-induced skin inflammation. Carcinogenesis 2013; 34: 397-405.
[20] Tsalkidou EG, Tsaroucha AK, Chatzaki E, Lambropoulou M, Papachristou F, Trypsianis G, Pitiakoudis M, Vaos G and Simopoulos C. The effects of apigenin on the expression of Fas/FasL apoptotic pathway in warm liver ischemia-reperfusion injury in rats. Biomed Res Int 2014; 2014: 157216.